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(54) Title: A LOW PHOSPHORUS ANIMAL FEED CONTAINING 1α-HYDROXYLATED VITAMIN D COMPOUNDS

(57) Abstract

An animal feed containing 1 α-hydroxylated vitamin D compounds. The vitamin D compounds cause improved utilization of phosphorus, calcium, potassium, magnesium, zinc, iron and manganese in animal feed so as to minimize, or perhaps eliminate, the need for supplemental quantities of these minerals in an animal diet. In addition, low phosphorus containing animal feeds reduce the polluting effects on the environment since less phosphorus is excreted in the animal's feces which are then spread on agricultural land.

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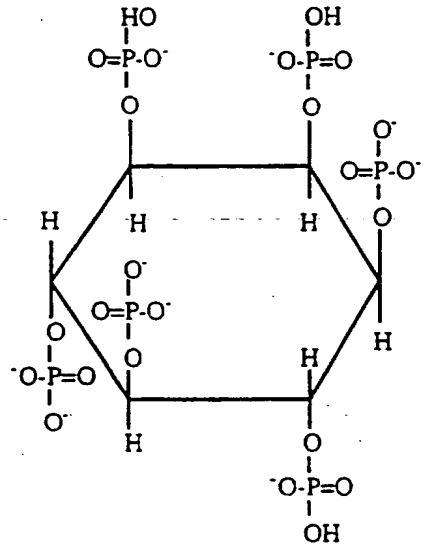
A LOW PHOSPHORUS ANIMAL FEED CONTAINING 1α-HYDROXYLATED VITAMIN D COMPOUNDS

Background and Summary of the Invention

Up to 80% of the phosphorus (P) present in plant foods and feeds exists as a complex of phytic acid (myoinositol hexaphosphate), hereinafter referred to as phytate. Phytate may structurally be illustrated by the following formula:

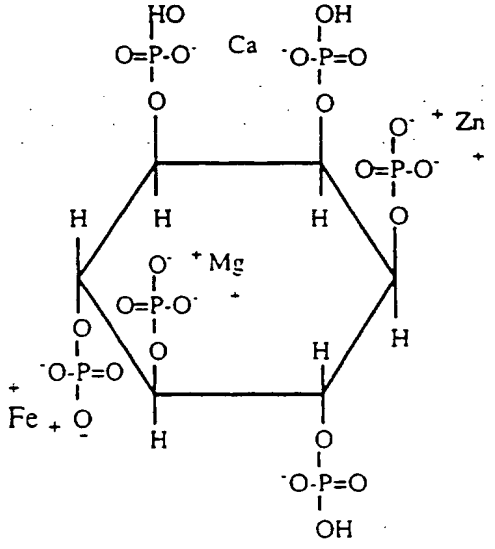


The P in phytate cannot be totally digested by simple-stomached animals, including humans, and it therefore passes through the gastrointestinal (GI) tract and is excreted in the feces. In animal nutrition, this is accounted for in diet formulation whereby 1.5 to 2.0% of an inorganic phosphate source is supplemented to meet the animal's minimal P requirement. Addition of inorganic P to poultry, swine, companion animal, and fish diets is expensive. It is often stated that supplemental P for these species is the third most expensive dietary ingredient, after energy and protein. The body requires P for formation of bones and teeth, for phospholipid (cell membrane structure) and nucleic acid (RNA, DNA) synthesis, for synthesis of ATP and other high-energy P compounds, and for proper acid-base balance in the body. Roughly 85% of the body P is in the

skeleton. Bone is comprised of 50% organic matrix (protein in the form of collagen, and lipid) and 50% inorganic material (mostly a Ca-P salt, i.e., hydroxyapatite).

Supplemental inorganic P is provided to animal diets in one of three feedgrade forms; dicalcium phosphate (18.5% P), monocalcium phosphate (21.5% P) or deflorinated phosphate (18.0% P). The combined total market for these products is estimated to be 675 million dollars per year in the U.S., Canada, Mexico, Western Europe and Japan. If one were to include South America, Eastern Europe, Asia, Africa, China, India, and Southeast Asia, (where market data are difficult to obtain), the total market for feed-grade phosphates could easily be expected to exceed 1 billion dollars annually. In North America, 50% of feed-grade phosphate consumed is used for poultry feeding. It has been discovered that use of a bioactive 1-α-OH vitamin D compound would reduce the need for supplemental P by up to 40%, and if combined with the enzyme phytase, could reduce the need by up to 50% to 60%.

Phytate complexes in plant foods and feeds (eg., cereal grains and by-products, beans) also bind cations such as calcium, potassium, magnesium, zinc, iron and manganese (Erdman, 1979) illustrated schematically as follows:



A bioactive feed additive that causes the utilization of P from phytate should also increase utilization of these elements as well. The present invention has established that 1-α-OH vitamin D compounds, preferably 1,25 dihydroxycholecalciferol and 1-α-OH cholecalciferol, increase the utilization of not only P but also zinc, iron and manganese. Thus, because these three trace elements are always added in supplemental form to diets for swine, poultry and companion animals (as feed-grade ZnO or $ZnSO_4 \cdot H_2O$; $FeSO_4 \cdot H_2O$; MnO or $MnSO_4 \cdot H_2O$) use of a bioactive 1-α-OH vitamin D compound would lower, or perhaps eliminate, the need for supplemental quantities of these mineral salts in a practical-type grain-oilseed meal diet.

By replacing up to 0.75% of the diet as a P supplement and up to 0.10% as trace mineral salts, the remaining diet would contain more usable energy. Thus, grain-oilseed meal diets generally contain about 3,200 kcal metabolizable energy per kilogram of diet, and mineral salts supply no metabolizable energy. Removal of the unneeded minerals and substitution with grain would therefore increase the usable energy in the diet.

Currently, phytase is being used in much of Europe and Asia to reduce P pollution. The use level, however, is 600 units per kilogram diet, but this level was selected because of cost of the enzyme and not because 600 units will maximize phytate utilization. In contrast it has been discovered via the present investigation that at least 1200 units/kg diet is required to maximize phytate utilization in chicks fed a corn-soybean meal diet (Table 1). However, use of a bioactive 1-α-OH vitamin D compound in accordance with the present invention would reduce the need to feed expensive levels of phytase. (Table 5)

Animal producers are forced to feed high P diets because of the phytate content of diets. This increases P in the excreta waste products (both feces and urine). Excess P from animal, as well as human waste, is generally spread on the soil, where a

portion of it gets washed into ground water and then into ponds, streams, rivers, lakes and oceans. Too much P in water stimulates growth of algae, and algae take up considerable oxygen. This robs marine life of the oxygen they need to grow, reproduce and thrive.

In many parts of Europe and Asia, P pollution has become such a problem and concern that penalties in the form of stiff financial fines are imposed on livestock producers who spread too much P-laden manure on the soils. Because of this, much of Europe now uses a microbial phytase product (BASF), even though this product (which also hydrolyzes phytate) is very expensive, in fact too expensive to be cost effective (at 600 units/kg diet) as a feed additive in the U.S. at the present time. Many U.S. soils are being described as "P saturated", thus resulting in a greater concentration of P in soil leachates. High-P water leachate in areas such as the Chesapeak Bay has been blamed for excessive algae growth and increased fish kills in bay waters (Ward, 1993). In Europe, the feed industry group FEFANA issued a position paper in 1991 entitled "Improvement of the Environment". They proposed that P in manure from livestock production should be reduced by 30% (Ward, 1993). The limits of P that can be applied to soils in Europe have been discussed by Schwarz (1994). Accordingly, it is estimated that use of a 1-α-OH vitamin D compound that is active in increasing phosphorus utilization in accordance with the present invention, could cut the P content of animal waste products by up to 40%.

Initial work focused on use of 1,25 dihydroxycholecalciferol (1,25-$(OH)_2D_3$) in the absence or presence of 1200 units of microbial phytase (BASF). Edwards (1993) showed that 1,25-$(OH)_2D_3$ is effective in improving P utilization from phytate-bound P, and Biehl et al (1995) confirmed his results. Moreover, both studies showed that 1,25-$(OH)_2D_3$ works additively with

microbial phytase in releasing P from dietary phytate complexes. It seems likely that 1,25-$(OH)_2D_3$ exerts is effects in two ways: (a) the 1,25 compound likely increases the activity of intestinal phytases or phosphatases that hydrolyze phytate (Pileggi et al. 1955; Maddaiah et al, 1964) and (b) the 1,25 compound is known to stimulate phosphate transport (Tanka and DeLuca, 1974), facilitating transport of P from GI tract to plasma and hence bone.

Under normal dietary circumstances, cholecalciferol (vitamin D_3) that is added to a diet gets absorbed from the GI tract and is transported via blood to the liver where the liver enzyme 25-hydroxylase acts on the compound to cause formation of 25-OH D_3. This compound is the normal blood metabolite of cholecalciferol. A small portion of 25-OH D_3 undergoes a further hydroxylation step in the kidney, at the 1-α position, causing synthesis of the calciotropic hormone 1,25-$(OH)_2D_3$. Because 1,25-$(OH)_2D_3$ is expensive to synthesize and because oral 25-OH D_3 is not the active form in phosphate absorption, it was proposed that 1-α-OH D_3 would be an effective compound for increasing phosphate utilization. It has been discovered that 1α-hydroxylated vitamin D compounds and particularly 1-α-OH D_3 will be absorbed from the GI tract and then be transported to the liver where 25-hydroxylase would act upon it to bring about synthesis of 1,25-dihydroxylated compounds and particularly 1,25-$(OH)_2D_3$. A portion of these compounds would then be transported back to the GI tract where they would activate intestinal phosphate absorption. The net effect would be an increased utilization of P (also Zn, Fe, Mn and Ca) from the phytate complex.

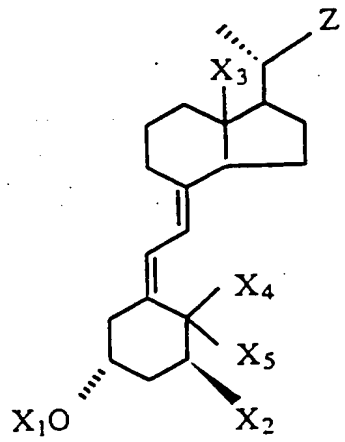
In summary, the potential benefits of the present invention include (1) reduction in the need for inorganic P supplements for animal (including fish) diets; (2) reduction in P pollution of the environment; (3) reduction or possible

elimination of the need for supplemental Zn, Mn and Fe in animal diets; and (4) reduction of the quantity of phytase needed for maximal P utilization from feeds.

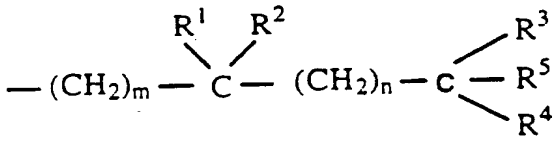
Detailed Description of the Preferred Embodiment

As used in the description and in the claims, the term hydroxy-protecting group signifies any group commonly used for the temporary protection of hydroxy functions, such as for example, alkoxycarbonyl, acyl, alkylsilyl, and alkoxyalkyl groups, and a protected hydroxy group is a hydroxy function derivatized by such a protecting group. Alkoxycarbonyl protecting groups are groupings such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, benzyloxycarbonyl or allyloxycarbonyl. The term "acyl" signifies an alkanoyl group of 1 to 6 carbons, in all of its isomeric forms, or a carboxyalkanoyl group of 1 to 6 carbons, such as an oxalyl, amlonyl, succinyl, glutaryl group, or an aromatic acyl group such as benzoyl, or a halo, nitro or alkyl substituted benzoyl group. The word "alkyl" as used in the description or the claims, denotes a straight-chain or branched alkyl radical of 1 to 10 carbons, in all its isomeric forms. Alkoxyalkyl protecting groups are groupings such as methoxymethyl, ethoxyethyl, methoxyethoxymethyl, or tetrahydrofuranyl and tetrahydropyranyl. Preferred alkylsilyl protecting groups are trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, and analogous alkylated silyl radicals.

The vitamin D compounds useful in the present treatment are 1α-hydroxylated vitamin D compounds, preferably 1α-hydroxycholecalciferol and 1α,25-dihydroxycholecalciferol. The vitamin D compounds of this type are characterized by the following general structure:



where X_1 may be hydrogen or a hydroxy-protecting group, X_2 may be hydroxy, or protected hydroxy, X_3 may be hydrogen or methyl, X_4 and X_5 each represent hydrogen or taken together X_4 and X_5 represent a methylene group, and where Z is selected from Y, -OY, $-CH_2OY$,-C≡CY and-CH=CHY, where the double bond may have the cis or trans stereochemical configuration, and where Y is selected from hydrogen, methyl, $-CR_5O$ and a radical of the structure:



where m and n, independently, represent integers from 0 to 5, where R^1 is selected from hydrogen, hydroxy, protected-hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3 and R^4, independently, is selected from hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an

oxo group, or an alkylidene group, $=CR_2R_3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 presents hydrogen, hydroxy, protected-hydroxy, or C_{1-5} alkyl.

The above compounds may be administered alone or in combination with other feed additive agents. The above vitamin D compounds or combinations thereof can be readily administered either by mixing them directly into animal feed or separately from the feed by separate oral dosage, by injection or by transdermal means or in combination with other 1α-hydroxylated vitamin D compounds, the proportions of each of the compounds in the combination being dependent upon the particular problem being addressed and the degree of response desired, are generally effective to practice the present invention. In poultry, amounts in excess of about 10 micrograms per day or the combination of that compound with other 1α-hydroxylated vitamin D compounds, are generally unnecessary to achieve the desired results, may result in hypercalcemia, and may not be an economically sound practice. It should be understood that the specific dosage administered in any given case will be adjusted in accordance with the specific compounds being administered, the problem to be treated, the condition of the subject and the other relevant facts that may modify the activity of the compound or the response of the subject, as is well known by those skilled in the art. In general, either a single daily dose or divided daily dosages may be employed, as is well known in the art.

If administered separately from the animal feed, dosage forms of the various compounds can be prepared by combining them with non-toxic pharmaceutically acceptable carriers to make either immediate release or slow release formulations, as is well known in the art. Such carriers may be either solid or liquid such as, for example, corn starch, lactose, sucrose, peanut

oil, olive oil, sesame oil and propylene glycol. If a solid carrier is used the dosage form of the compounds may be tablets, capsules, powders, troches or lozenges or top dressing as micro-dispersable forms. If a liquid carrier is used, soft gelatin capsules, or syrup or liquid suspensions, emulsions or solutions may be the dosage form. The dosage forms may also contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, etc. They may also contain other therapeutically valuable substances.

The present invention also relates to an animal feed composition and method of compounding an animal feed utilizing a 1α-hydroxylated vitamin D compound to lower the dietary requirement of phosphorus in the animal feed. The 1α-hydroxylated vitamin D compounds suitable for this use have been previously described herein. The amount of a phosphorus supplement (18.5%P) that may be incorporated with the feed may be reduced to about 0.9% from about 1.9% on a dry weight basis. This is a significant reduction from the normal amount of phosphorus supplement incorporated in animal feed compositions of about 1.5% to about 2.5%. This beneficial reduction in phosphorus is a direct result of the incorporation of a 1α-hydroxylated vitamin D compound in the animal feed.

The animal feed may be any protein-containing organic meal normally employed to meet the dietary requirements of animals. Many of such protein-containing meals are typically primarily composed of corn, soybean meal or a corn/soybean meal mix. For example, typical commercially available products fed to fowl include Egg Maker Complete, a poultry feed product of Land O' Lakes AG Services, as well as Country Game & Turkey Grower a product of Agwa, Inc. Both of these commercially available products are typical examples of animal feeds with which the present 1α-hydroxylated vitamin D compounds may be incorporated to reduce the amount of supplemental phosphorus.

zinc, manganese and iron intake required in such compositions. Thus, any type of protein-containing organic meal may be utilized as the base mix to which the 1α-hydroxylated vitamin D compounds and reduced supplemental phosphorus, zinc, manganese and iron amounts of the present invention may be incorporated.

The present invention is applicable to the diet of numerous animals, which herein is defined as including mammals, fowl and fish. In particular, the diet may be employed with commercially significant mammals such as pigs, cattle, sheep, goats, laboratory rodents (rats, mice, hamsters and gerbils), fur-bearing animals such as mink and fox, and zoo animals such as monkeys and apes, as well as domestic mammals such as cats and dogs. Typical commercially significant fowl include chickens, turkeys, ducks, geese, pheasants and quail. Commercially formed fish such as trout would also benefit from the diet disclosed herein.

In a method of compounding feed for animals in accordance with the present invention, the 1α-hydroxylated vitamin D compounds utilized is incorporated with the animal feed in an amount of from about 5μg/kg to about 40μg/kg feed on a dry weight basis. The feed mixture is then fed as a mash or as formed into desired discrete shapes for further processing and packaging. In general, these discrete shapes may be pellets, blocks or briquettes formed by known extrusion and/or compacting techniques. The particular processing technique utilized does not affect the performance of the 1α-hydroxylated vitamin D compounds in the animal feed mixture. The present invention is more specifically described by the following examples, which are meant to be illustrative only.

Chick Efficacy Trials

A. Procedures:

The best measure of P (or Ca) activity in animals fed a P-deficient diet is total bone ash. In the present bioassay system, young chicks (8 d of age) are fed a corn-soybean meal diet containing 0.6% Ca and 0.43% total P, but an estimated 0.10% bioavailable P. The required levels of Ca and P for chicks of this age are 1.0% Ca and 0.45% available P (i.e., nonphytate P). Calcium is kept at 0.6% instead of 1.0% in our diet because excess Ca in the presence of a severe available P deficiency causes anorexia.

Generally speaking, three or four pens of three or four chicks per pen are placed on each dietary treatment. They are fed the experimental diets free choice for 12 d in wire-screened battery pens located in a environmentally controlled animal room with constant (fluorescent) lighting. At assay termination on d 20 posthatching, chicks are killed by cervical dislocation and the left tibia is quantitatively removed. Bones are stripped of adhering tissue, dried for 24 h at 100°C, weighed and then dry ashed for 24 h at 600°C (muffle furnace). The portion remaining after ashing is entirely inorganic matter. The weight of ash (mineral matter) as a percent of dry bone weight is percent ash (mineral, and mostly Ca and P) in the bone. Percent ash multiplied by dry bone weight gives total bone ash in milligrams. Tibia ash reflects the degree of ash (or bone mineralization) in the entire skeleton. Our 20-d-old crossbred chicks (New Hampshire x Columbian) fed a diet adequate in Ca and P generally have percent bone ash values of 45%.

For assessment of Zn and Mn bioavailability, bone content of Zn and Mn are the established criteria, but growth responses are also used for assessment of Zn bioavailability (Wedekind et al, 1992; Halpin and Baker, 1986). For assessment of Zn or Mn bioavailability, the tibiae are dried at 100°C for 24 h, weighed,

and then dry ashed at 600°C for another 24 h. The dried ash is then wet ashed with HNO_3 and H_2O_2. Zinc and manganese are then quantified using atomic absorption spectrophotometry (Wedekind et al, 1992). In research involving Zn, Mn or Fe (hemoglobin assay) bioavailability, the chicks are fed a pretest diet (0 to d-8 posthatching) that is deficient in Zn, Mn or Fe. This depletes stores of these trace elements. The experiments are then carried out in stainless-steel chick batteries equipped with stainless-steel feeders and waterers. Deionized water is available free choice. These steps are taken to avoid Zn, Mn or Fe contamination from the environment, equipment and drinking water.

B. Results:

The basal diet for the first experiment was designed to be severely deficient in available P (most coming from phytate-bound P) but adequate to excess in vitamin D_3, and marginal in both Zn and Mn (i.e., no supplemental Zn or Mn in diet). Increases in bone ash would indicate enhanced GI absorption of P, and increases in bone Zn and Mn would indicate enhanced GI absorption of Zn and Mn (Chung and Baker, 1990; Wedekind et al, 1992; Halpin and Baker, 1986; Baker et al, 1986). As shown in Table 1, growth rate was increased ($P<0.05$) 17% by 0.10% P addition, 20% by 1200 U phytase addition, 15.5% by 1,25-$(OH)_2D_3$ addition, and 25% by the combination of phytase (1200 U) and 10.0 µg/kg 1,25-$(OH)_2D_3$. Bone ash, however, is the best measure of P bioavailability. Total bone ash (mg) was increased ($P<0.01$) 56% by 0.10% P addition (proving that P was severely deficient in the diet), 64% with 1200 U phytase, 60% by 1,25-$(OH)_2D_3$, and 98% by the combination of phytase and 1,25-$(OH)_2D_3$. Tibia Zn (µg) was increased ($P<0.01$) 55% by either 1200 U phytase or 10 µg/kg 1,25-$(OH)_2D_3$, but was increased

86% by the phytase-di-OH D_3 combination. Tibia Mn (µg) was increased ($P<0.01$) 63% by phytase, 85% by di-OH D_3 and 123% by the phytase-di-OH D_3 combination.

Data in Table 2 show results of a second efficacy trial. The basal diet for this trial was made adequate in Ca, and also was fortified with normal (safety factor) levels of Mn and Zn. It was thus singly deficient in available P. Bone ash was markedly depressed in chicks fed the P-deficient negative control diet. In fact, bone ash percent was about 5% lower (30.4% in Exp. 1, 25.5% in Exp. 2) in these chicks, a reflection of the high ratio of Ca to available P. Efficacy was again demonstrated for both phytase and 1,25-$(OH)_2D_3$. Moreover, the diet containing both phytase and 1,25-$(OH)_2D_3$ produced both ash values that were not far from those achieved with a P adequate diet (diet 5).

Data in Table 3 show results of a classic Zn efficacy trial. The basal diet was singly deficient in Zn (the NRC 1994 Zn requirement is 40 ppm) so that even with 10 ppm Zn addition, the diet was still Zn deficient. Marked efficacy was observed for both phytase and 1,25-$(OH)_2D_3$, and additivity was again evident for the combination.

Having shown conclusively that 1,25-$(OH)_2D_3$ is markedly efficacious in utilization of P, Zn and Mn, a trial was next conducted to test the efficacy of 1-α-OH D_3. These results are shown in Table 4. A linear ($P<0.01$) growth response occurred when 1-α-OH D_3 doses between 0 and 20 µg/kg were supplemented. Tibia ash likewise increased ($P<0.01$) markedly when 1-α-OH D_3 was added to the diet. Total tibia ash (mg) was 69% higher in chicks fed the diet with 20 µg/kg 1-α-OH D_3 than in those fed the unsupplemented basal diet. A dose of 40 µg/kg 1-α-OH D_3 was efficacious, and certainly nontoxic, but the 20 µg/kg dose maximized the response attributable to P release from phytate.

Data in Table 5 verify the synergism between the combination of microbial phytase and 1,25-$(OH)_2D_3$. Also, the results demonstrate that when phytase (600 vs. 1200 units) doses are compared in the presence of 10 µg/kg 1,25-$(OH)_2D_3$, 600 units of phytase are as effective as 1200 units in improving phytate-P utilization. This finding when compared to the data of Exp. 1 (Table 1) indicates that the phytase supplementation level required for maximum response can be cut in half if a supplemental bioactive 1-α-OH vitamin D compound is also included in the diet. In fact, only 300 units of phytase produced a marked response in the presence of 1,25-$(OH)_2D_3$.

Data in Table 6 show that synergism exists between 1-α-OH D_3 and phytase. Thus, 20 µg/kg 1-α-OH D_3 combined with 1200 units of phytase increased total bone ash by 107% over that observed for the basal unsupplemented corn-soybean meal diet. Supplemental 1-α-OH D_3 alone increased bone ash by 74%, and supplemental phytase alone increased bone ash by 65%.

TABLE 1
Phytase and 1,25-Dihydroxycholecalciferol Increase Growth Rate and Bone Strength of Young Chicks Fed a Phosphorus-Deficient Diet (Exp. 1)[1]

Diet[2]	Weight gain (g)	Gain feed (g/kg)	Avail. P intake (mg)	Tibia data[3] Weight (mg)	Ash (%)	Ash (mg)	Zn (µg/g)	Zn (µg)	Mn (µ/g)	Mn (µg)
				Phosphorus titration[4]						
0	193	644	300	667	30.4	203				
0.05% P	200	640	468	717	35.4	254				
0.10% P	226	657	688	827	38.3	317				
				Phytase titration						
0	193	644	300	667	30.4	203	142	95	2.32	1.55
300 µ phytase[5]	202	647	312	729	33.9	247	145	105		
600 µ phytase	206	661	312	735	35.8	263	159	117	2.66	1.96
900 µ phytase	224	664	338	805	38.2	308	171	137		
1200 µ phytase	231	679	340	848	39.3	333	173	147	3.00	2.53
				Factorial						
1. 0	193	644	300	667	30.4	203	142	95	2.32	1.55
2. 1200 U phytase[5]	231	679	340	848	39.3	333	173	147	3.00	2.53
3. 10 µg/kg DiOH-D_3[6]	223	683	326	816	39.6	324	179	147	3.52	2.87
4. As 2 + 3	241	707	340	932	43.1	402	190	177	3.85	3.46
Pooled SEM	3.3	6.7		14	.5	7.0	4.1	4.4	.10	.08

Table 1 footnotes on next page.

TABLE 1 FOOTNOTES

[1]Data represent means per chick of four replicate pens of four female chicks during the period 8 to 20-d posthatching; average initial weight was 82 g.

[2]The basal corn-soybean meal diet (23% CP) contained 0.10% available P and 0.60% Ca. Neither Mn or Zn were provided as supplements to this basal diet. The diet was adequate to excess in vitamin D_3, containing 1000 IU of supplemental cholecalciferol per kg of diet (25µg/kg).

[3]Dry weight basis.

[4]Graded doses of P from KH_2PO_4.

[5]Phytase obtained from BASF Corp., Parsippany, NJ 07054. One unit (U) of phytase is defined as the quantity of enzyme required to liberate 1 µmol of inorganic P per minute from 1.5 mmol/L sodium phytase at pH 5.5 and 37°C. Phytase was added from a premix (Natuphos® 5.000 BASF) that contained 5,000 U of phytase activity per gram.

[6]Dihydroxycholecalciferol (DiOH-D_3) obtained from Hoffman-LaRoche, Inc., Nutley, NJ. DiOH-D_3 was dissolved in propylene glycol to make a solution of 10µg/ml. The desired volume of DiOH-D_3 solution for each diet involved was then dissolved in petroleum ether, which was then premixed with basal diet and subsequently added to the completed diet for mixing.

TABLE 2

Effects of Phytase and 1,25 Dihydroxycholecalciferol on Performance and Bone Characteristics of Chicks Fed Diets Deficient in Phosphorus and Adequate in Calcium (Exp. 2)[1]

Diet	12-d weight gain (g)	Gain feed (g/kg)	Avail. P intake (mg)	Tibia data[2]						
				Weight (mg)	Ash (%)	Ash (mg)	Zn (µg/g)	Zn (µg)	Mn (µ/g)	Mn (µg)
1. Basal (B)[3]	172	649	266	598	25.5	152	146	88	3.29	1.98
2. B + 1200µ phytase[4]	218	678	322	780	37.5	292	219	171	4.82	3.76
3. B + 10µg/kg diOH-D_3[5]	201	686	293	698	36.1	253	199	139	5.29	3.69
4. As 2 + 3	219	702	311	847	42.5	360	216	183	5.86	4.96
5. B + .45% P[6]	244	688	1952	959	45.3	435	189	181	3.54	3.39
Pooled SEM	4.4	7.1		22	.45	9.5	5	6	.13	.12

[1]Data represent mean values per chick of four replicates (pens) of three chicks during the period 8 to 20-d posthatching; average initial weight was 83 g.

[2]Intact left tibia (dry basis).

[3]The basal corn-soybean meal diet (23% CP) contained .10% available P and 1.0% Ca. Both Mn and Zn were provided as supplements to this basal diet (50 mg/kg of each) such that the basal diet was singly deficient in available P.

[4]See footnote 5 of Table 1.

[5]See footnote 6 of Table 1.

[6]Provided from KH_2PO_4.

TABLE 3
Efficacy of Phytase and 1,25 Di-OH-D_3 in Chicks Fed a Zn-Deficient Diet (Exp. 3)[1]

Diet[2]	12 days gain (g)	Tibia Zn (µg/g)	Tibia Zn (µg)
1. Basal diet	169	44.7	34.2
2. As 1 + 1200 U phytase	209	62.2	54.9
3. As 1 + 10µg/kg Di-OH-D_3	201	60.3	53.1
4. As 2 + 3	241	88.4	88.7
5. As 1 + 5 ppm Zn ($ZnSO_4 \cdot 7H_2O$)	210	61.5	54.2
6. As 2 + 10 ppm Zn ($ZnSO_4 \cdot 7H_2O$)	236	73.7	71.1
Pooled SEM	8		2.7

[1]Data are means of four pens, each containing four male chicks weighing 84.5 g at day 8 posthatching; 12-d feeding period in stainless-steel batteries with chicks receiving deionized water. During the 8-d pretest period, chicks were fed a low Zn soybean meal diet.

[2]Soy concentrate-dextrose diet containing 13 ppm Zn.

TABLE 4
Dietary Addition of 1-α-hydroxycholecalciferol Increases Phytate-Phosphorus Utilization (Exp. 4)[1]

Dietary Level of 1-α-OH-D_3 (μg/kg)	12-d weight gain[3] (g)	Gain feed[3] (g/kg)	Tibia Data[3]		
			Weight (mg)	Ash (%)	Ash (mg)
0	228[b]	645[b]	724[c]	33.0[b]	238[c]
10	255[a]	676[a]	917[b]	38.9[a]	356[b]
20	266[a]	681[a]	992[a]	40.5[a]	402[a]
40	255[a]	677[a]	878[b]	41.1[a]	361[b]
Pooled SEM	3.6	6.5	21	.75	7.6

[1]Means of three pens of four chicks during the period 8 to 20 days posthatching.

[2]Added to a corn-soybean meal diet (23% CP) containing adequate vitamin D-3, 0.60% Ca and 0.43% P (0.10% estimated available P).

[3]Means within columns with unlike superscript letters are significantly ($P < 0.5$) different.

TABLE 5
Performance and Bone Ash of Chicks Fed 1,25-Dihydroxycholecalciferol in the Absence or Presence of Three Levels of Microbial Phytase (Exp. 5)[1]

Dietary addition[2]	Weight gain[3] (g)	Food intake (g)	Tibia data[3] Weight (mg)	Ash (%)	Ash (mg)
1. None	203[c]	314[c]	672[c]	32.9[4]	238[d]
2. 10µg/kg di-OH-D_3	234[b]	338[b]	825[b]	42.2[c]	348[c]
3. As 2 + 300 U phytase	244[a]	349[a,b]	881[a,b]	42.5[b,c]	375[b]
4. As 2 + 600 U phytase	251[a]	361[a]	903[a]	43.9[a,b]	396[a,b]
5. As 2 + 1200 U phytase	252[a]	356[a]	886[a]	44.7[a]	396[a,b]
Pooled SEM	3.6	4.6	20	0.5	9.0

[1]Data are means for four pens of four female chicks that were fed the experimental diets during the period 8 to 20 d posthatching; average initial weight was 93 g. Means in columns with different superscripts letters are significantly different ($P < 0.05$).

[2]The basal diet (Table 1) contained, by analysis, 0.43% P (0.10% estimated available P), 0.63% Ca and 23% crude protein.

[3]Dry-weight basis.

TABLE 6
Evaluation of 1-α-Hydroxycholecalciferol With and Without Phytase on Phosphorus Utilization[1]

Dietary addition	Weight gain	Food intake	Tibia data		
			Weight	Ash	Ash
	g	g	mg	g/100 g	mg
1. None	195[c]	306[b]	634[c]	29.1[c]	185[c]
2. 0.10g P/100g (KH_2PO_4)	239[a,b]	355[a]	801[b]	38.7[b]	310[b]
3. 1200 U phytase	245[a,b]	356[a]	795[b]	38.5[b]	306[b]
4. 20 µg/kg 1-α-OH-D_3	235[b]	343[a]	787[b]	40.9[a]	321[b]
5. As 3 + 4	253[a]	363[a]	897[a]	42.7[a]	384[a]
Pooled SEM	5.5	6.6	18	0.7	11

[1]Data are means of three pens of four female chicks that are fed the experimental diets during the period 8 to 20 d posthatching; average initial weight was 88 g. Means in columns with different superscript letters are significantly different ($P < 0.05$).

[2]The basal corn-soybean meal diet contained, by analysis, 0.43 g P/100 g (0.10 g/100 g estimated nonphytate P), 0.63 g Ca/100 g and 23.9 g CP/100 g.

[3]Dry-weight basis.

We claim:

1. A method of compounding feed for animals, comprising the steps of:

providing an animal feed comprising a diet containing about 0.5 % to 1.9% of the diet as an inorganic phosphorus supplement;

incorporating with said diet an effective amount of a 1α-hydroxylated vitamin D compound to form a feed mixture; and

forming said feed mixture into a discrete shape.

2. The method of claim 1 wherein said discrete shape is formed by extruding said mixture.

3. The method of claim 1 wherein said discrete shape is formed by compacting said mixture.

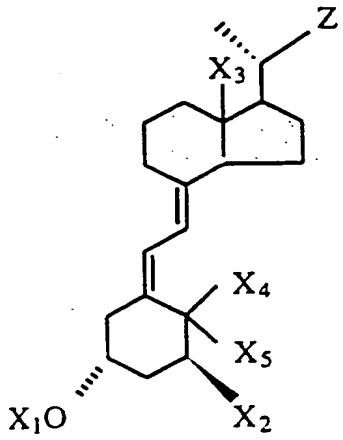
4. The method of claim 1 wherein said effective amount of the 1α-hydroxylated vitamin D compound comprises about 5μg/kg to about 40μg/kg of diet.

5. The method of claim 1 further including the step of incorporating an effective amount of phytase with said diet.

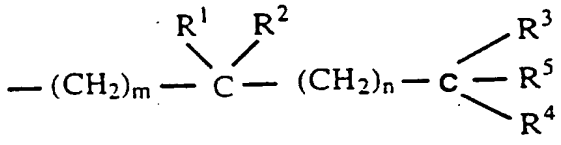
6. The method of claim 5 wherein said effective amount of phytase comprises from about 300 units to about 1,200 units.

7. The method of claim 1 wherein said effective amount of phytase comprises about 600 units per kilogram of diet.

8. The method of claim 1 wherein said 1α-hydroxylated vitamin D compound is characterized by the following general structure:



where X_1 may be hydrogen or a hydroxy-protecting group, X_2 may be hydroxy, or protected hydroxy, X_3 may be hydrogen or methyl, X_4 and X_5 each represent hydrogen or taken together X_4 and X_5 represent a methylene group, and where Z is selected from Y, -OY, $-CH_2OY$, $-C\equiv CY$ and $-CH=CHY$, where the double bond may have the cis or trans stereochemical configuration, and where Y is selected from hydrogen, methyl, $-CR_5O$ and a radical of the structure:



where m and n, independently, represent integers from 0 to 5, where R^1 is selected from hydrogen, hydroxy, protected-hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3 and R^4, independently, is selected from hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or

branched, and optionally bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR_2R_3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 presents hydrogen, hydroxy, protected-hydroxy, or C_{1-5} alkyl.

9. The method of claim 1 wherein the vitamin D compound is 1α-hydroxyvitamin D_3.

10. The method of claim 1 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

11. An animal feed composition comprising:

a diet containing about 0.5% to 1.9% of an inorganic phosphorus supplement; and

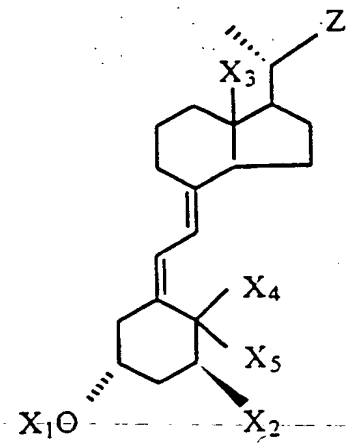
an effective amount of an 1α-hydroxylated vitamin D compound for utilizing phosphorus from phytate complexes in said diet.

12. The composition of claim 11 wherein said effective amount of the 1α-hydroxylated vitamin D compound comprises about 5μg/kg to about 40μg/kg of diet.

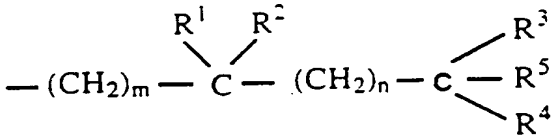
13. The composition of claim 11 further including from about 300 units to about 1,200 units phytase in said diet.

14. The composition of claim 11 further including about 600 units phytase per kilogram of diet.

15. The composition of claim 11 wherein said 1α-hydroxylated vitamin D compound is characterized by the following general structure:



where X_1 may be hydrogen or a hydroxy-protecting group, X_2 may be hydroxy, or protected hydroxy, X_3 may be hydrogen or methyl, X_4 and X_5 each represent hydrogen or taken together X_4 and X_5 represent a methylene group, and where Z is selected from Y, -OY, $-CH_2OY$, $-C\equiv CY$ and $-CH=CHY$, where the double bond may have the cis or trans stereochemical configuration, and where Y is selected from hydrogen, methyl, $-CR_5O$ and a radical of the structure:



where m and n, independently, represent integers from 0 to 5, where R^1 is selected from hydrogen, hydroxy, protected-hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3 and R^4, independently, is selected from hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally bear a hydroxy or protected-hydroxy

substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR_2R_3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 presents hydrogen, hydroxy, protected-hydroxy, or C_{1-5} alkyl.

16. The composition of claim 11 wherein the vitamin D compound is 1α-hydroxyvitamin D_3.

17. The composition of claim 11 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

18. A method of minimizing dietary requirements of phosphorus in animals comprising the steps of:

feeding a diet containing about 0.5% to 1.9% of an inorganic phosphorus supplement to an animal; and

feeding with said diet an effective amount of a 1α-hydroxylated vitamin D compound for utilizing phosphorus from phytate complexes in said diet.

19. The method of claim 18 wherein said 1α-hydroxylated vitamin D compound is fed as a top dressing on said diet.

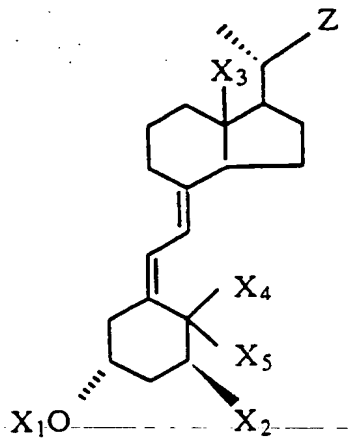
20. The method of claim 18 wherein said effective amount of the 1α-hydroxylated vitamin D compound comprises about 5µg/kg to about 40µg/kg of diet.

21. The method of claim 18 further including the step of incorporating an effective amount of phytase with said diet.

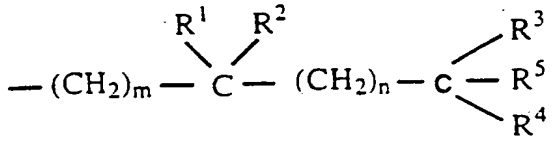
22. The method of claim 18 wherein said effective amount of phytase comprises from about 300 units to about 1,200 units in said diet.

23. The method of claim 18 wherein said effective amount of phytase comprises about 600 units per kilogram of diet.

24. The method of claim 18 wherein said 1α-hydroxylated vitamin D compound is characterized by the following general structure:



where X_1 may be hydrogen or a hydroxy-protecting group, X_2 may be hydroxy, or protected hydroxy, X_3 may be hydrogen or methyl, X_4 and X_5 each represent hydrogen or taken together X_4 and X_5 represent a methylene group, and where Z is selected from Y, -OY, $-CH_2OY$, -C≡CY and-CH=CHY, where the double bond may have the cis or trans stereochemical configuration, and where Y is selected from hydrogen, methyl, $-CR_5O$ and a radical of the structure:



where m and n, independently, represent integers from 0 to 5, where R^1 is selected from hydrogen, hydroxy, protected-hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3 and R^4, independently, is selected from hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an

oxo group, or an alkylidene group, $=CR_2R_3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 presents hydrogen, hydroxy, protected-hydroxy, or C_{1-5} alkyl.

25. The method of claim 18 wherein the vitamin D compound is 1α-hydroxyvitamin D_3.

26. The method of claim 18 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

27. A method of reducing the deleterious polluting effects of phosphorus on the environment comprising the steps of:

feeding a diet containing about 0.5% to 1.9% of an inorganic phosphorus supplement to an animal;

feeding with said diet an effective amount of a 1α-hydroxylated vitamin D compound to utilize phosphorus from phytate complexes in said diet;

collecting excreta waste products containing reduced phosphorus levels produced by the animal; and

spreading said waste products on soil.

28. The method of claim 27 wherein said 1α-hydroxylated vitamin D compound is fed as a top dressing on said diet.

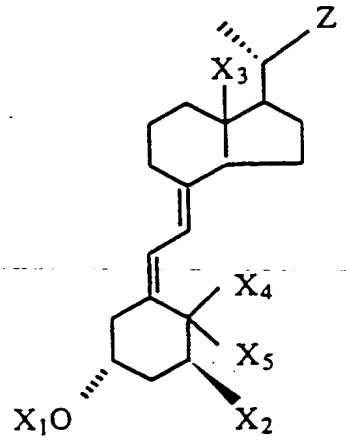
29. The method of claim 27 wherein said effective amount of the 1α-hydroxylated vitamin D compound comprises about 5μg/kg to about 40μg/kg of diet.

30. The method of claim 27 further including the step of incorporating an effective amount of phytase with said diet.

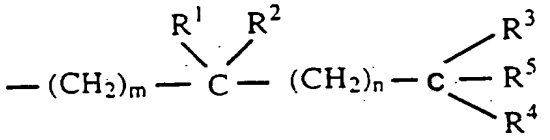
31. The method of claim 27 wherein said effective amount of phytase comprises from about 300 units to about 1,200 units in said diet.

32. The method of claim 27 wherein said effective amount of phytase comprises about 600 units per kilogram of diet.

33. The method of claim 27 wherein said 1α-hydroxylated vitamin D compound is characterized by the following general formula:



where X_1 may be hydrogen or a hydroxy-protecting group, X_2 may be hydroxy, or protected hydroxy, X_3 may be hydrogen or methyl, X_4 and X_5 each represent hydrogen or taken together X_4 and X_5 represent a methylene group, and where Z is selected from Y, -OY, $-CH_2OY$,-C≡CY and-CH=CHY, where the double bond may have the cis or trans stereochemical configuration, and where Y is selected from hydrogen, methyl, $-CR_5O$ and a radical of the structure:



where m and n, independently, represent integers from 0 to 5, where R^1 is selected from hydrogen, hydroxy, protected-hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3 and R^4, independently, is selected from hydrogen, fluoro.

trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR_2R_3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 presents hydrogen, hydroxy, protected-hydroxy, or C_{1-5} alkyl.

34. The method of claim 27 wherein the vitamin D compound is 1α-hydroxyvitamin D_3.

35. The method of claim 27 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

36. A method of degrading phytate complexes in animal feed, comprising the steps of:

providing an animal feed comprising a diet containing phytate complexes that bind desirable cations;

incorporating with said diet an effective amount of a 1α-hydroxylated vitamin D compound to form a feed mixture; and

feeding said feed mixture to an animal.

37. The method of claim 36 wherein said desirable cations are selected from calcium, potassium, magnesium, zinc, iron, manganese and phosphorus.

38. The method of claim 36 wherein said 1α-hydroxylated vitamin D compound is fed as a top dressing on said diet.

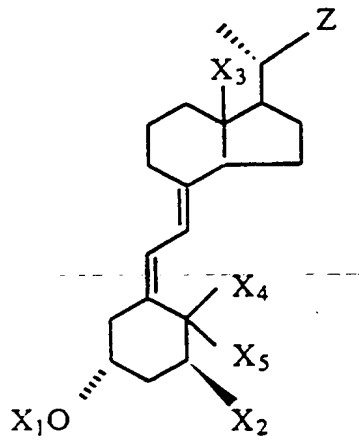
39. The method of claim 36 wherein said effective amount of the 1α-hydroxylated vitamin D compound comprises about 5μg/kg to about 40μg/kg of diet.

40. The method of claim 36 further including the step of incorporating an effective amount of phytase with said diet.

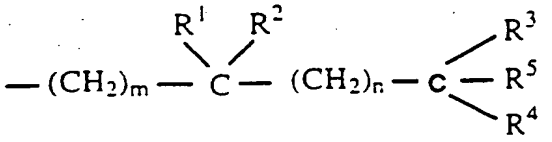
41. The method of claim 36 wherein said effective amount of phytase comprises from about 300 units to about 1,200 units in said diet.

42. The method of claim 36 wherein said effective amount of phytase comprises about 600 units per kilogram of diet.

43. The method of claim 36 wherein said 1α-hydroxylated vitamin D compound is characterized by the following general formula:



where X_1 may be hydrogen or a hydroxy-protecting group, X_2 may be hydroxy, or protected hydroxy, X_3 may be hydrogen or methyl, X_4 and X_5 each represent hydrogen or taken together X_4 and X_5 represent a methylene group, and where Z is selected from Y, -OY, $-CH_2OY$, $-C\equiv CY$ and $-CH=CHY$, where the double bond may have the cis or trans stereochemical configuration, and where Y is selected from hydrogen, methyl, $-CR_5O$ and a radical of the structure:



where m and n, independently, represent integers from 0 to 5, where R^1 is selected from hydrogen, hydroxy, protected-hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3 and R^4,

independently, is selected from hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR_2R_3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 presents hydrogen, hydroxy, protected-hydroxy, or C_{1-5} alkyl.

44. The method of claim 36 wherein the vitamin D compound is 1α-hydroxyvitamin D_3.

45. The method of claim 36 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

INTERNATIONAL SEARCH REPORT

In ..ational Application No
PCT/US 96/01021

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23K1/16 A61K31/59

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 19759 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC.) 14 October 1993 see page 6, paragraph 2 - page 7, paragraph 2 see claims 1,2,5-11,14,16	11-45
Y	---	1,8-10
Y	ZEITSCHRIFT FÜR VERSUCHSTIERKUNDE, vol. 27, no. 3/4, 1985, pages 163-168, XP002001500 ERLING TVEDEGAARD: "Absorption of calcium, magnesium and phosphate during chronic renal failure and the effect of vitamin D in rabbits" see page 163, paragraph 2	1,8-10

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 April 1996	09. 05. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (- 31-70) 340-2040, Tx. 31 651 epo nl, Fax (- 31-70) 340-3016	Dekeirel, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF NUTRITION, vol. 125, no. 9, 1995, pages 2407-2419, XP002001501 ROBERT R. BIEHL ET AL.: "1-alpha-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets" cited in the application see the whole document	11-45
X	POULTRY SCIENCE, vol. 73, no. 8, 1994, pages 1312-1326, XP002001502 KEVIN D. ROBERSON ET AL.: "Effects of 1,25-dihydoxycholecalciferol and phytase on zinc utilization in broiler chicks" see page 1312, Abstract see page 1325, column 1, paragraph 1	36-43,45
A	POULTRY SCIENCE, vol. 69, no. 3, 1990, pages 426-432, XP002001503 R.H. HARMS ET AL.: "Some observations on the influence of vitamin D metabolites when added to the diet of commercial laying hens" see page 426, column 2, paragraph 2 - page 427, column 1, paragraph 1 see page 427; table 1	1,8-10
A	EP,A,0 383 116 (F. HOFFMANN-LA ROCHE AG) 22 August 1990 see column 4, line 18 - line 55 see claims 1,2	1,8-10
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